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Fructooligosaccharides integrity after atmospheric cold plasma and high-pressure processing of a functional orange juice

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ABSTRACT

In this study, the effect of atmospheric pressure cold plasma and high-pressure processing on the prebiotic orange juice was evaluated. Orange juice containing 7 g/100 g of commercial fructooligosaccharides (FOS) was directly and indirectly exposed to a plasma discharge at 70 kV with processing times of 15, 30, 45 and 60 s. For high-pressure processing, the juice containing the same concentration of FOS was treated at 450 MPa for 5 min at 11.5 °C in an industrial equipment (Hyperbaric, model: 300). After the treatments, the fructooligosaccharides were qualified and quantified by thin layer chromatography. The organic acids and color analysis were also evaluated. The maximal overall fructooligosaccharides degradation was found after high-pressure processing. The total color difference was < 3.0 for high-pressure and plasma processing. citric and ascorbic acid (Vitamin C) showed increased content after plasma and high-pressure treatment. Thus, atmospheric pressure cold plasma and high-pressure processing can be used as non-thermal alternatives to process prebiotic orange juice.

1. Introduction

Thermal technologies are still the standard processing technology for fruit juices preservation. However, thermal processing may cause changes in functional molecules, vitamins and other nutrients (Vervoort et al., 2011). Orange juice is one of the most consumed juice due to its vitamin C content (Barba, Esteve, Tedeschi, Brandolini, & Frígola, 2013). Orange juice pasteurization can cause off-flavors and the degradation of the product's quality due to non-enzymatic browning (Patil, Bourke, Frias, Tiwari & Cullen, 2009). Because of that, non-thermal technologies have been seeking to produce foods with a minimum of nutritional, physicochemical and organoleptic changes (Barba, Parniakov, et al., 2015; Barba, Terefe, Buckow, Knorr, and Orlien, 2015). For functional foods, these technologies must also be able to preserve the compounds responsible for their functionality.

Among the available non-thermal technologies, atmospheric pressure cold plasma (ACP) and high-pressure processing (HPP) are alternatives for food preservation maintaining the fresh-like characteristics of foods (Ramos, Miller, Brandão, Teixeira & Silva, 2013). The use of ACP and HPP on fruits and vegetables presented a positive effect on pathogens and enzyme inactivation (Misra et al., 2014; Ziuzina, Patil, Cullen, Keener, & Bourke, 2014; Patterson, 2005). Direct and indirect ACP has been used as an alternative for microbial inactivation in solid and liquid foods (Misra et al., 2014; Niemira, 2012; Surowsky, Schlüter, & Knorr, 2015; Ziuzina et al., 2014). Montenegro, Ruan, and Ma (2002) reported that nonthermal plasma discharges applied directly to the food product, proved effective in reducing the number of *Escherichia coli* O157: H7 cells in apple juice, by up to 7 log units. Orange juice samples inoculated with either *Staphylococcus aureus ATCC6538*, *Escherichia coli ATCC8039*, or *Candida albicans ATCC 10231* were ACP treated at 20 kV at low temperature (22 to 25 °C) for 12, 8, and 25 s, respectively, and the numbers of each microorganism decreased > 5 log units. The native microorganisms (non-inoculated) in orange juice treated with low-temperature plasma for 10 and 12 s, were all inactivated (Shi et al., 2011b, 2011a).

High-pressure processing (HPP), involves applying very high pressures (100–1000 MPa) for a short time (up to 20 min) to packaged food using water as a pressure transmission medium to (Aganovic et al.,

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2015; Andrés, Villanueva, & Tenorio, 2016). The high-pressure treatment is also defined as high pressure hydrostatic pressure (HHP) treatment and high-pressure homogenization (HPH) treatment (Qiu, Li, Chen, Liu, & Yin, 2014). HPP processing of orange juice resulted in non-detectable microbial counts immediately after the processing (Bull et al., 2004). There are other published works on HPP effect in orange juice (Zulueta, Barba, Esteve & Frígola, 2013; Barba, Esteve, and Frígola, 2012; Esteve, Barba, Palop, & Frígola, 2009). However, a single study was published regarding the HPP effect in FOS (Keenan, Brunton, Butler, Wouters, & Gormley, 2011). To the best of our knowledge, there is no published data showing the advantages and limitations of ACP applied to fructooligosaccharides (FOS), the most studied and used functional carbohydrate in the food industry (Dominguez, Rodrigues, Lima, & Teixeira, 2013).

Due to previous reports on FOS degradation in fruit juices submitted to thermal treatment (Klewicki, 2007; Matusek, Merész, Le, & Örsi, 2009), the evaluation of a non-thermal treatment for fruit juice processing fortified with FOS is mandatory to attest its suitability for such kind of food. Also, the processing efficiency is usually dependent on the food matrix. Thus, each food matrix should be evaluated separately (Zinoviadou et al., 2015).

The present study evaluated the effect of ACP and HPP on the integrity of functional fructooligosaccharides (FOS), citric and ascorbic acid (Vitamin C) and on the orange juice color. The amount of FOS added to the juice was based on the recommended daily intake (RDI) of foods containing prebiotic compounds, which should provide at least 3 g of FOS in solid foods or 1.5 g in liquid foods (ANVISA). Besides, the FOS consumption should not exceed 30 g per day in products ready for consumption.

2. Materials and methods

2.1. Prebiotic orange juice preparation

A pure orange juice (Sqeez©, Fruit Juices Ltd., Ireland), obtained from concentrated orange juice, without sugars or preservatives addition was purchased from a local supermarket (Dunnes, Dublin - Ireland). The juice is commercially sterile and stored at room temperature in carton packages. Once opened the juice lasts 4 days under refrigeration. This kind of juice was chosen to avoid any enzyme or microbial interference on the results. The juice containing prebiotic ingredients was prepared by adding 7 g/100 g of purified and food-grade fructooligosaccharides (ORAFTI© P95, Beneo GmbH, Mann, Germany). The pH of the juice was determined by direct measurement in a 420A potentiometer, (Orion Research Inc., Beverly, MA., US) calibrated with buffer solutions of pH 4.0, 7.0 and 10.0.

2.2. Atmospheric pressure cold plasma treatment (ACP)

The plasma treatment was carried out using a plasma generator system (DBD-ACP), model: Phenix 6CP120/60-7.5 (ISRE Instrumentation Sales & Rentals). The system consists of a variable high voltage transformer with an input voltage of 230 V at 50 Hz and a maximum high voltage output of 70 kV at 50 Hz. The two 15-cm-diameter aluminum disc electrodes were separated by a polypropylene container, which served as a sample holder and as a dielectric barrier with a wall thickness of 1.2 mm. The distance between the two electrodes was 22 mm and equal to the height of the container. An InfiniVision 2000 X-Series Oscilloscope (Agilent Technologies Inc., Santa Clara, CA, USA) monitored the voltage. All experiments were performed at 70 kV peak to peak at ambient air and atmospheric pressure conditions.

A volume of 20 mL of orange juice containing 7 g/100 g of FOS was transferred to an open Petri dish. The Petri dish was placed in a polypropylene box that was sealed with a polymeric film of 50 μm thickness (Cryovac BB3050), which served as an additional layer of dielectric barrier (Pankaj et al., 2014). Samples were treated with atmospheric pressure cold plasma (ACP) for of 15, 30, 45 and 60 s and different exposure kinds: direct plasma field (in) and indirect plasma field (out). In the direct plasma field, the sample is placed under the electrodes receiving the generated field. In the indirect plasma field, the sample is placed beside the electrodes receiving the field in an indirect way as presented by Ziuzina et al. (2014). The treatment times were selected based on a previous study carried out by Ziuzina et al. (2014) for *E. coli* inactivation, where 20 s of direct and 45 s of indirect plasma treatment was enough to bacterial inactivation for pasteurization purposes (7 log reduction). The treated samples of prebiotic orange juice were stored at room temperature for 24 h before opening the container. The ACP was carried out inside a laminar flow hood under aseptic conditions. A control sample, containing the same concentration of FOS dissolved in distilled water was prepared, packed and treated as done for the prebiotic orange juice. The use of water as solvent aimed to eliminate the low pH effect of the orange juice on FOS integrity, allowing the evaluation of the ACP effect without any interaction with the food matrix components.

2.3. High-pressure processing

High-pressure processing (HPP) was carried out using industrial equipment (Hyperbaric, model: 300). Prebiotic orange juice was transferred to 250 mL polyethylene bottles and vacuum sealed in polypropylene bags for the high-pressure processing. A control sample, containing the same concentration of FOS dissolved in water was prepared, packed and treated in the same way that the prebiotic orange juice. The processing was done at the industrial conditions: 5 min at 450 MPa as recommended by HPP Tolling Business (Dublin, Ireland) for fruit juices. The desired pressure was reached in < 4 s, and the depressurization was instantaneous. The temperature in the pressure chamber was controlled at 11.5 °C during the complete processing. The studies on HPP in fluid foods processing are usually done in small equipment (vessel capacity up to 3 L) using small volumes (Barba et al., 2013; Barba, Cortés, Esteve, & Frígola, 2012; Bull et al., 2004). In the present study, an industrial equipment was used (vessel capacity of 300 L), and real HPP conditions were applied. After the treatment, the juice quality was evaluated.

2.4. Carbohydrate analysis

2.4.1. Characterization of FOS degree of polymerization

FOS were analyzed by Thin Layer Chromatography (TLC). The prebiotic orange juice samples were diluted (1:2), filtered, using glass fiber pre-filters AP25 13 mm diameter (Merck Millipore Ltd.), and cleaned on a C-18 SPE cartridge. The samples were analyzed by TLC, using silica gel on TLC plates (SIGMA-ALDRICH 20 × 20 cm, 60 Å medium pore diameter; product number: 99570-25EA). Samples of 1 μL were applied on the plate at 1 cm from the bottom and at a separation distance of 1.0 cm from each other. The plates were disposed in the TLC chamber pre-conditioned at room temperature. The solvent system used to separate the carbohydrate mixture was an n-butanol/2-propanol/H_2O (10:5:4 [vol/vol/vol]) mixture (Shiomi, Onodera & Sakai, 1997). The TLC plate was irrigated by the solvent system three times. The plate was air dried in a hood after each solvent ascent. To visualize the carbohydrates, a fine spray containing 1-butanol/water (80:10 v/v), phosphoric acid (6.78 mL), urea (3 g) and ethanol (8 mL) in 100 mL was used. After air drying in a hood, the plates were oven heated at 120 °C for 10 min to render the spots visible. For the quantification of the oligosaccharides, a TLC scanner CAMAG 4 20 × 20 cm densitometer was used. The wavelength used was 450 nm. The Planar WinCATS Chromatography Manager software was used to handle the data. The analyses were performed in triplicate. The results were expressed as relative concentration, calculated according to Eq. (1).

$$\text{Relative concentration (\%)} = \frac{A_{DPi}}{A_t} \times 100 \quad (1)$$

where:

A_i chromatographic area of an individual FOS DP (i = 3 to 7)

A_t sum of the individual chromatographic areas ($\sum_{i=3}^{i=7} A_i$)

2.5. Color

The color of the samples was measured using a colorimeter (Color Quest XE Hunter Lab, Northants, UK). The instrument operates on CIELAB (L*, a* and b*) and it was calibrated with a white standard (L* = 93.97, a* = 0.88 and b* = 1.21) standard. The parameters L^* (lightness), a^* (redness) and b (yellowness) were measured and used to calculate the chromaticity, the hue angle and the total color difference (ΔE), according to Eqs. (2), (3) and (4), respectively. The reference to the total color difference was the non-processed juice (control).

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

$$\text{Hue} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

2.6. Organic acids

High-Performance Liquid Chromatographic (HPLC) analysis was used to quantify the organic acids (Yuk, Sampedro, Fan, & Geveke, 2014). The prebiotic orange juice samples were diluted (1:4) and filtered, using glass fiber prefilters AP25 13 mm diameter (Merck Millipore Ltd.) to remove the suspended solids. After that, the sample was cleaned on a C-18 SPE cartridge and filtered through a cellulose acetate membrane (0.45 μM, 13 mm of diameter). The analysis was carried out in Agilent HPLC system (Agilent Technologies 1260 Infinity) equipped with a pump system and a UV-DAD detector. The organic acids were separated on an Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad) at 50 °C and 210 nm. The isocratic elution was performed with 0.01 M sulfuric acid in deionized water as mobile phase for 30 min at 0.6 mL/min. The analyses were performed in triplicate.

2.7. Statistical analysis

Statistical analyzes were performed using the statistical software Statistica (StatSoft) version 10.0. The results were compared by Tukey test at a 95.0% confidence level ($p < 0.05$). To evaluate the effect of each processing on FOS degradation, a multifactorial ANOVA was performed where factor 1 was the kind of the treatment and factor 2 the FOS degree of polymerization. All experiments were carried out in replicate and all analysis in triplicate.

3. Results and discussion

3.1. Effect of ACP and HPP on fructooligosaccharides

The thin layer chromatography (TLC) allowed the quantification of the prebiotic fructooligosaccharides concentration along with their degree of polymerization (DP). The results were expressed as the relative concentration obtained by densitometry (Robyt, 2000). The target oligosaccharides were kestose (DP3), nystose (DP4), 1-fructofuranosyl-nystose (DP5), and FOS with DP6 and DP7. To illustrate the results, Fig. 1 shows the TLC analysis of the fructooligosaccharides in water and orange juice after direct exposure to plasma treatment.

According to the manufacturer (BENEO-ORAFT), the FOS used in this study (P95) presents about 6 g/100 g of mono and di-saccharides. These sugars spots on TLC are darker for orange juice due to their higher amount in fruit juices. Figs. 2 and 3 shows the relative amounts of FOS in water and orange juice, after direct and indirect plasma exposure. The percentage of DP-7, DP-6, 1-FFN (DP5), nystose (DP4) and kestose (DP3) in water are similar to the percentages of these fructooligosaccharides in orange juice.

The sample treated by direct plasma exposition showed a slight degree of change in the FOS DP during the treatment (Fig. 2). The FOS profile in water after ACP indirect exposure (Fig. 3a) showed a fast change in the first 15 s of processing and after that has presented a lower degree of change compared to the direct exposure (Fig. 2a). The FOS DP in orange juice sample (Fig. 2b) presented more changes along the treatment time compared to the FOS dissolved in water (Figs. 2a and 3a). These changes could be attributed to the interaction between the FOS and the juice compounds. Despite the changes observed, the overall amount of oligosaccharides in the juice after the ACP treatment was well preserved. Besides, the DP changes were small and did not compromise the juice functionality.

Unlike some reported studies where plasma treatment degraded carbohydrates (Park et al., 2007; Benoit et al., 2011), FOS in orange juice were well preserved. Fig. 4 shows the fructooligosaccharides in water (control) and orange juice after the high-pressure processing (HPP). The percentage of each degree of polymerization in water was similar to ones found in the processed juice. A slight decrease on DP7 and DP6 was accompanied by a slight increase on kestose (DP3), suggesting a small depolymerization due to HPP treatment. The profile obtained after HPP treatment was close to the obtained by the indirect exposition to ACP for the FOS diluted in water (Fig. 3a).

Multifactor ANOVA was used to compare the three processes: HPP, direct ACP and indirect ACP, allowing a better comparison of the trends presented by each technology. As shown in Fig. 5, the process applying ACP direct exposition of the sample showed the lowest overall change in the degree of polymerization of FOS. As a result, the highest concentration of high degree FOS was observed after direct exposition to ACP, in both water and orange juice samples. The use of HPP showed the highest change in the FOS DP. Compared to the direct exposure to ACP, HPP presented the lowest amount of DP7 and the highest amount of DP3, denoting a higher degree of depolymerization both for the water and orange juice samples. Fisher LSD test confirmed the statistically significant difference between the results obtained between direct ACP and HPP for DP7 and DP3.

Regarding the FOS in water sample (Fig. 5a), the use of ACP under indirect exposition showed to decrease the amount of DP7 in a similar way observed for HPP, but the depolymerization was less intense, denoted by the increase of DP6 and DP5. The depolymerization observed during HPP was more intense, and an increase in DP4 and DP3 was greater, with a consequent reduction in the amounts of DP7, DP6, and DP5.

When the matrix changed to orange juice (Fig. 5b), the use of ACP under indirect exposition showed a much smaller decrease for DP7. While a certain degree of depolymerization existed, it was lesser than the one observed in the FOS + water system and much lower than the one observed using HPP. Fisher LSD test confirmed the statistically significant difference, for DP7 and DP3, between the results obtained between indirect ACP and HPP, and between direct ACP and HPP. No statistically significant difference between direct and indirect ACP were observed for orange juice samples.

The results obtained are in agreement with previous studies for HPP juice processing regarding bioactive compounds, which reported a great retention of bioactive compounds aside the preservation of the physical-chemical properties of fruit juices after high-pressure processing (Chen et al., 2015; Patras, Brunton, Pieve, Butler & Downey et al. 2009).

Matusek et al. (2009) and Klewicki (2007) studied the stability of functional ingredients during thermal pasteurization at lower pH values. These authors concluded that the FOS was highly susceptible to hydrolysis in pasteurization conditions of fruit juices and drinks.

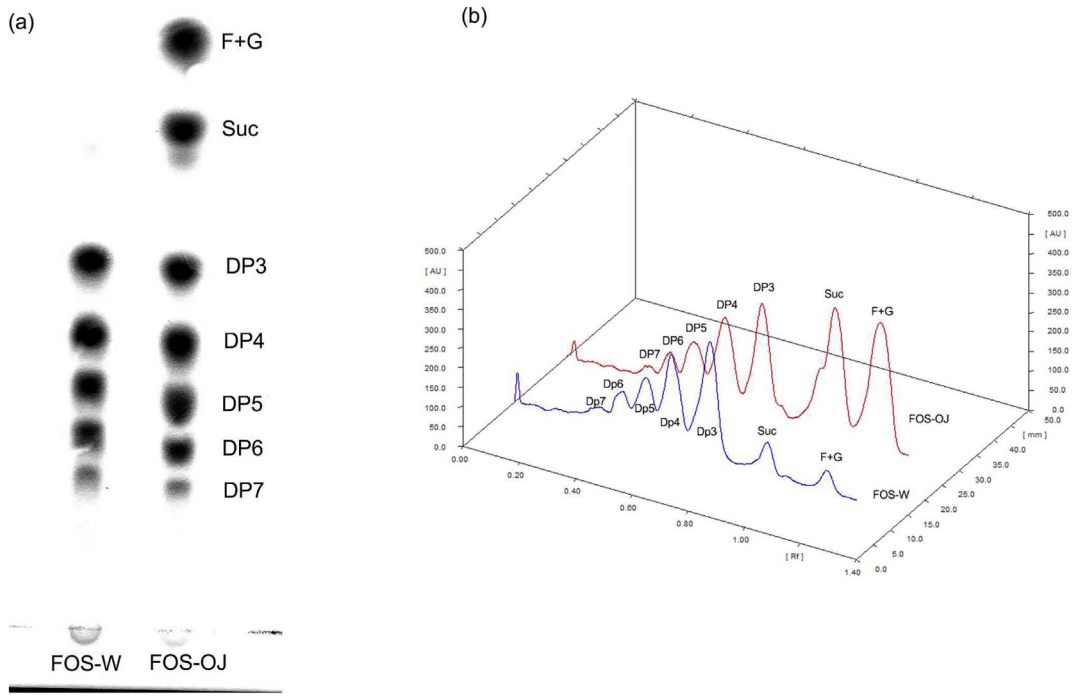

Fig. 1. Degree of polymerization of the fructooligosaccharides after direct exposure of plasma treatment. TLC plate (a) and densitometer chromatogram (b). FOS-W means fructooligosaccharides dissolved in water (control) and FOS-OJ fructooligosaccharides added to orange juice.

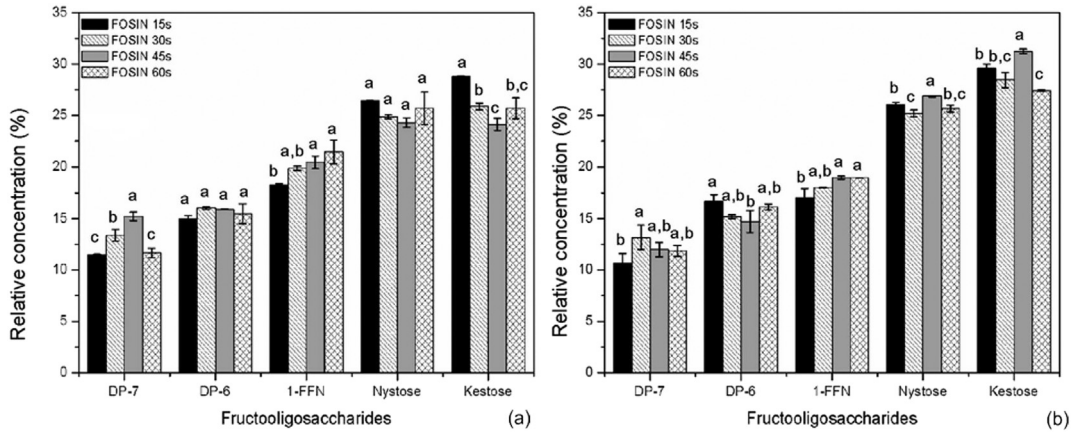

Fig. 2. FOS relative concentration in water (a) and in orange juice (b) after direct plasma exposure (IN). DP means the degree of polymerization. Results are present as mean ± standard deviation of three replicates. The effect of each processing time on the FOS relative concentration was compared by Tukey test. Different letters for the same treatment time means that the samples are statistically different ($p \leq 0.05$).

According to Matusek et al. (2009), the FOS hydrolysis at 70–80 °C was considerable, and the total FOS amount could be halved in 1 to 2 h. For higher temperatures, the effect was worst since all the oligomers were degraded in 1 to 1.5 h at 90–100 °C. Keenan et al. (2011) reported FOS hydrolysis of 26.2 g/100 g after thermal treatment and, of 17.1 g/100 g after HPP treatment of prebiotic apple puree. On the other hand, non-significant FOS degradation was reported by Gomes et al. (2017) for cranberry juice after HPP and ultrasound processing. FOS in water was also stable after ultrasound processing, and ultrasound followed by HPP (Alves Filho et al., 2016). Thus, not only the processing but also the food matrix affects the FOS integrity.

Galactooligosaccharides (GOS) showed higher stability compared to FOS when submitted to industrial fruit juices pasteurization. The lower the pH and the longer the pasteurization time, the higher was the FOS hydrolysis. The pasteurization of a blackcurrant drink (pH 3.1; 95 °C/30 s + 84 °C/10 min) causes the loss of about 50 g/100 g of tetrasaccharide (DP4) and up to 30 g/100 g of trisaccharides (DP3).

There is no other published study on FOS degradation by plasma. Thus, it is not possible to compare the results obtained herein with other plasma treated juices regarding FOS integrity. Almeida et al. (2015) studied the glucooligosaccharides degradation by plasma and ozone in orange juice. Ozone and plasma direct exposure result in overall hydrolysis of 22 g/100 g and 21 g/100 g, respectively. The lower hydrolysis (12 g/100 g) was found after direct plasma exposure. In the presented study, the estimated overall FOS degradation is presented in Table 1. Less hydrolysis was observed for FOS in water compared to FOS in orange juice. Comparing the results obtained in the present study with the reported by Almeida et al. (2015) for glucooligosaccharides, the plasma treatment (direct and indirect exposure) resulted in less FOS hydrolysis. The HPP treatment resulted in less FOS degradation than the reported by Keenan et al. (2011) for apple puree. The results corroborate with Zinoviadou et al. (2015) who studied the ultrasound treatment of fruit juices and concluded that the processing effect is dependent not only on the processing intensity and duration

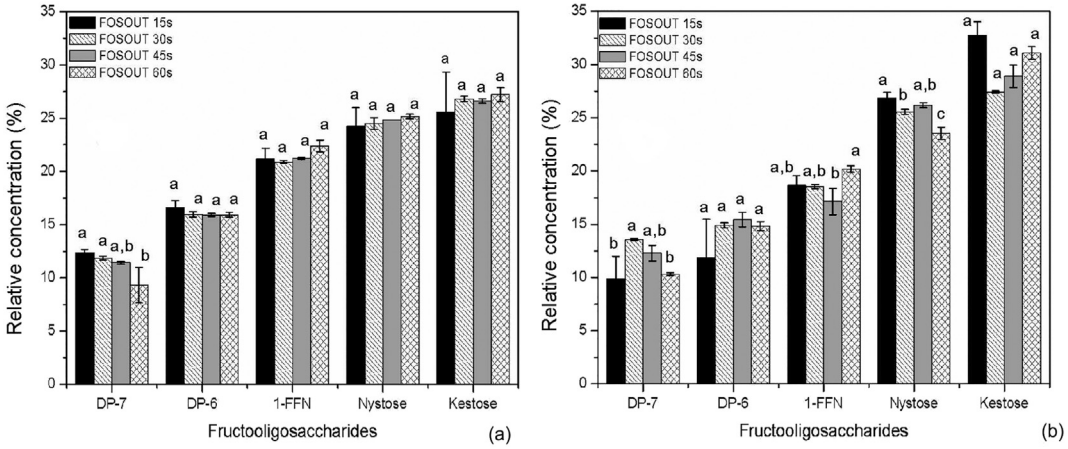

Fig. 3. FOS relative concentration in water (a) and in orange juice (b) after indirect plasma exposure (OUT). DP means the degree of polymerization. Results are present as mean ± standard deviation of three replicates. The effect of each processing time on the FOS relative concentration was compared by Tukey test. Different letters for the same treatment time means that the samples are statistically different ($p \leq 0.05$).

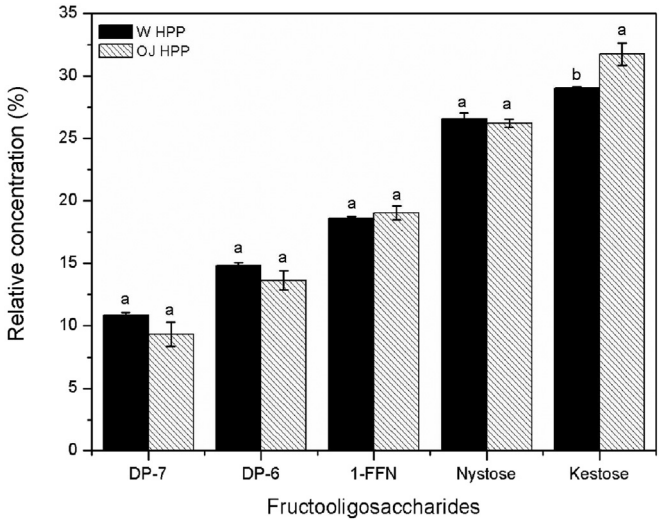

Fig. 4. FOS relative concentration in water and orange juice after high-pressure processing. DP means the degree of polymerization. Results are present as mean ± standard deviation of three replicates. The effect of each process time on the FOS relative concentration was compared by Tukey test. Means with different letters, the samples are statistically different ($p \leq 0.05$).

but also on the food matrix.

Although some changes were found in the FOS DP after ACP and HPP processing, they are much lower than the reported for thermal processing, making these technologies promising for orange juice pasteurization regarding the FOS preservation.

Table 1
Overall FOS hydrolysis after non-thermal treatment.

Non-thermal treatment	FOS in water (%)	FOS in orange juice (%)
Plasma IN	0.5	10.6
Plasma Out	1.7	15.2
HPP	11.2	21.4

3.2. Color parameters

Table 2 shows the color analysis of the ACP and HPP treated orange juice. The control sample (reference) was the non-treated juice containing FOS. For The ACP, the L parameter, which indicates the luminosity of the product, presented values form 55.96 ± 0.01 (non-treated juice) to 56.00 ± 0.01. The results showed statistical differences due to the low standard deviation. However, the highest difference between the samples was lower than 0.5% ($\Delta L_{max} = 0.16$), which has no practical application since the consumer would not perceive such small difference.

A slight change in the Chroma and Hue values of ACP treated samples were also observed. Again, the results were statistically different due to the low standard deviation. The maximum differences were < 3% for Chroma value ($\Delta C_{max} = 0.75$) and < 2% for hue value and ($\Delta h_{max} = 1.74$). Thus, despite the instrumental statistical difference the small change in the L, Chroma, and Hue value does not compromise the product acceptance. The total color difference (ΔE)

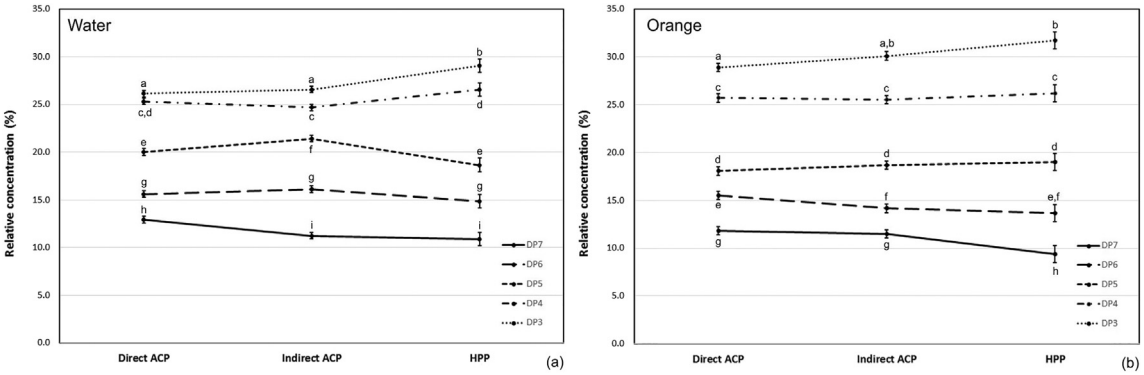

Fig. 5. Treatment effect on FOS relative concentration. The effect of each treatment was obtained by multifactorial ANOVA. Means with different letters are statistically different ($p \leq 0.05$).

Table 2
Color parameters of the orange juice after non-thermal treatment.

	L*	Chroma	h°	ΔE
Direct exposure				
Orange juice (control)	55.96 ± 0.01^{d}	30.58 ± 0.02^{a}	95.29 ± 0.02^{a}	0
15	56.10 ± 0.07^{b}	30.54 ± 0.01^{b}	94.28 ± 0.02^{b}	0.46 ± 0.01^{c}
30	56.02 ± 0.07^{b}	30.59 ± 0.03^{a}	94.13 ± 0.01^{c}	0.95 ± 0.01^{b}
45	56.03 ± 0.01^{c}	29.83 ± 0.01^{d}	93.97 ± 0.02^{d}	1.08 ± 0.01^{a}
60	56.16 ± 0.01^{a}	30.52 ± 0.02^{b}	93.55 ± 0.01^{e}	1.09 ± 0.02^{a}
Indirect exposure				
Orange juice (control)	55.96 ± 0.01^{e}	30.58 ± 0.02^{e}	95.29 0.02^{a}	0
15	56.33 ± 0.01^{a}	31.21 ± 0.01^{a}	94.76 ± 0.01^{b}	0.54 ± 0.01^{d}
30	56.15 ± 0.01^{d}	31.10 ± 0.02^{b}	94.46 ± 0.02^{c}	0.70 ± 0.02^{c}
45	56.22 ± 0.03^{b}	30.83 ± 0.01^{c}	94.05 ± 0.03^{d}	0.88 ± 0.01^{b}
60	56.18 ± 0.02^{c}	29.93 ± 0.01^{d}	94.02 ± 0.01e	1.09 ± 0.03^{a}
HPP				
Orange juice (control)	55.96 ± 0.01^{d}	30.58 ± 0.02^{a}	95.29 ± 0.02^{a}	0.0
Prebiotic orange juice	58.49 ± 0.19^{b}	41.71 ± 0.17^{b}	86.17 ± 0.02^{b}	2.1 ± 0.02

Results are mean ± standard deviation of three replicates. Different letters in the same column indicate that the samples are statistically different according to the Tukey test ($p \leq 0.05$).

was < 1.5 for all samples, which means that the color change is slightly noticeable (Barba, Esteve & Frígola, 2012; Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006).

The changes observed in the color parameters for HPP treated samples are higher than the observed for ACP treatment. However, the changes due to HPP were positive and improved the color of the final product. The L-value in HPP-treated sample increased from 55.96 ± 0.01 to 58.49 ± 0.19, which means that the sample became slightly lighter. The Chroma value increased from 30.58 ± 0.02 to 41.71 ± 0.17 indicating that the treated sample is more vivid than the non-treated sample. The hue value represents the characteristic color of the juice. This parameter decreased from 95.29 ± 0.02 to 86.17 ± 0.02. According to the color hunter space, the pure yellow corresponds to 90°. Orange juice characteristic color ranges from orange to yellow depending on the fruit variety and despite the Hue angle change, the treated juice color is within the expected color for orange juice. The total color difference obtained for HPP-treated sample (ΔE = 2.1) was higher than the observed for the plasma treated samples. The color change in HPP-treated sample is classified as noticeable (1.5 < ΔE < 3.0) according to Barba, Cortés, Esteve, and Frígola (2012) and Cserhalmi et al. (2006).

Barba, Cortés, Esteve & Frígola (2012) reported that total color change (ΔE) in HPP-treated orange juice–milk (200 to 400 MPa for 2 to 9 min) was significantly different from the unprocessed samples. However, the color difference was slightly higher than 3.0 only in the HPP treatment at 400 MPa for 9 min. Usually, ΔE values above 3 are the threshold for color change perception by the human eye (Pereira et al., 2013).

Despite the small variations in the instrumental color of the treated sample, the changes did not compromise the characteristic color of the juice for both non-thermal processing evaluated. HPP treatment enhanced the color, and the product became more vivid and lighter due to the increase of Chroma and L-value.

3.3. Effect of ACP and HPP on the juice pH

The pH values obtained for ACP and HPP treated orange juice containing FOS showed a pH drop to 3.90 to 4.00. The non-thermal treatments (ACP and HPP) decreased the juice pH values. The mean pH value obtained for the control was 4.43 ± 0.01 and the pH values for treated orange juice samples were around 3.90.

Orange juice pH depends on the variety and harvest and can be as low as 2.8 according to Sinclair, Bartholomew, and Ramsey (1945). This small decrease in the pH value is associated with the increase in the concentration of the main organic acids present in orange juice after the non-thermal processing as presented below. The results obtained are in agreement with the results reported by Bull et al. (2004), where a decrease of about 0.1 pH units was observed for HPP and pasteurized orange juice. Shi et al. (2011b, 2011a) also reported a slightly pH decrease for plasma treated orange juice.

3.4. Organic acids quantification by HPLC

The results are shown in Fig. 6 for citric acid and Fig. 7 for ascorbic acid (vitamin C). In HPP treatment, it was possible to observe a significant increase in the concentration of citric acid in the juice without FOS (10.60 ± 0.04 g/L) and in the juice with FOS (10.42 ± 0.02 g/L) when compared to the control (6.70 ± 0.06 g/L). The ACP treated samples presented similar behavior compared to HPP, with an increased concentration of citric acid along the treatment time.

For HPP treatment, the ascorbic acid (vitamin C) concentration in

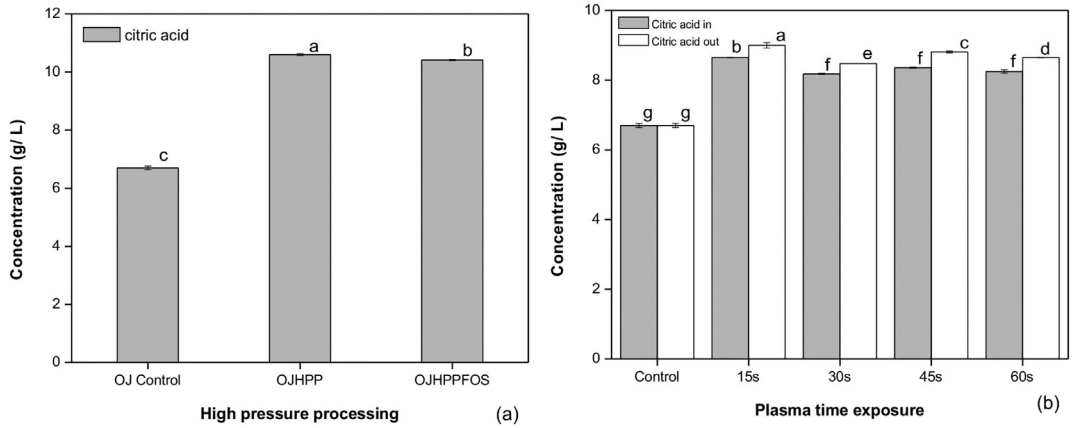

Fig. 6. Citric acid (g/L) in orange juice after high-pressure processing (a). OJ Control is the control sample (orange juice without FOS and non-submitted to the treatment). OJHPP is the processed juice without FOS and OJHPPFOS is the processed juice containing FOS. Citric acid (g/L) and after plasma treatment (b). Results are expressed as mean ± standard deviation of three replicates. Different letters means that the samples are statistically different according to the Tukey test ($p \leq 0.05$).

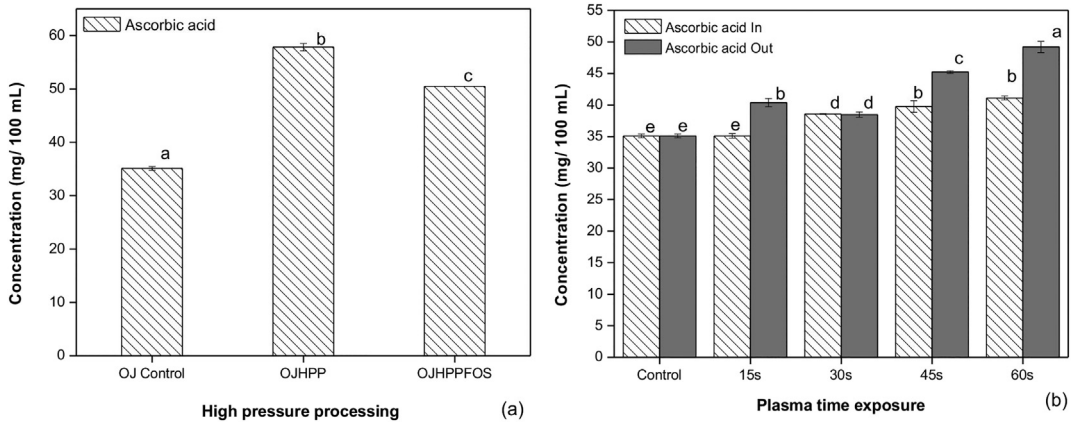

Fig. 7. Ascorbic acid (mg/100 mL) in orange juice after high-pressure processing (a). OJ Control is the control sample (orange juice without FOS and non-submitted to the treatment). OJHPP is the processed juice without FOS and OJHPPFOS is the processed juice containing FOS. Ascorbic acid (mg/L) after plasma treatment (b). Results are expressed as mean ± standard deviation of three replicates. Different letters means that the samples are statistically different according to the Tukey test ($p \leq 0.05$).

the juice with FOS (50.50 ± 0.04 mg/100 mL) and without FOS (57.84 ± 0.68 mg/100 mL) also increased after HPP treatment, compared to the control (from 35.10 ± 0.35 mg/100 mL). It was also observed a small increase in the concentration of ascorbic acid in plasma treated samples, reaching 41.11 ± 0.33 mg/100 mL after 60 s of ACP direct exposure and 49.21 ± 0.88 mg/100 mL after indirect ACP plasma exposure. Regarding the kind and time of ACP exposure, some small but statistically significant differences were found (Fig. 7).

Igual, García-Martínez, Camacho and Martínez-Navarrete (2010) reported that thermal treatments, such as conventional pasteurization, degraded organic acids in grapefruit. Their results showed that thermal treatment led to a significant decrease in citric acid (from 1.54 to 1.48 mg/100 g) and ascorbic acid (from 36 to 34.3 mg/100 g). Cserhalmi et al. (2006) also reported a decrease of 11 g/100 g in the vitamin C (ascorbic acid) content in raspberry juice after pulsed electric field (PEF) treatment. Velázquez-Estrada, Hernández-Herrero, Rüfer, Guamis-López, and Roig-Sagués (2013) evaluated the effect of the non-thermal process of ultra-high pressure on bioactive compounds of orange juice. Their results showed that the remaining content of L-ascorbic acid (vitamin C) after UHPH treated samples at any pressure was significantly higher than in the thermally pasteurized one. According to the authors, the vitamin C retained after HPP ranged from 89 to 98 g/100 g, depending on the pressure applied, while after the vitamin C retained after thermal treatment was 80 g/100 g. Shi et al. (2011b, 2011a) also reported a slight increase on orange juice acidity.

Khandpur and Gogate (2015) reported that orange juice submitted to ultrasound processing and ultrasound + UV light presented a better preservation of Vitamin C compared to thermal treatment. The authors reported vitamin C retained after the studied processing was 78 g/100 g for thermal treatment, 96 g/100 g for ultrasound and 92 g/100 g for thermal treatment. The comparison of the reported values evidence that thermal processing was the worst one compared to the HPP and ACP.

The effects of non-thermal treatments are usually non-linear and strongly dependent on the food matrix, and the processing conditions. The increase in some compounds was already reported after non-thermal processing due to several mechanisms such as cell disruption, dissociation of small-sized aggregated particles and also due to chemical reaction due to the reactive species formed in some non-thermal processing. HPP is a processing suitable for extraction and increase of bioactive compounds such as phenolic; carotenoids and minerals after HPP processing. Pressure level, the pressure holding time, liquid/solid ratio, type of solvent, and solvent concentration are the main parameter which affects the efficiency of HP extraction (Barba, Parniakov, et al., 2015). Cold plasma treated sour cherry Marasca juice present a higher amount of phenolic compounds (Elez Garofulić et al., 2015). According to the authors, the increase in phenolic compounds content in sour cherry Marasca juices could be a result of the plasma dissociation of small-sized aggregates or particles. Grzegorzewski, Ehlbeck, Schlüter, Kroh, and Rohn (2011) reported the increase on some phenolics in plasma treated lamb's lettuce due to the plasma-reactive species disintegration of cell membranes.

The results found in the present study, showed an increase in all organic acids. However, the food matrix studied in the present study contains FOS, which might affect the interaction between the food matrix compounds. Regarding the HPP processing, the use of industrial equipment allowed to control the temperature at low levels (~11.5 °C) avoiding any thermal effect. The increase of organic acids is consistent with the pH drop previously reported. The effects observed herein, agree with other published studies where the increase in some compounds was attributed to cell disruption, dissociation of small cell aggregates or particles among other mechanisms usually reported for non-thermal processing.

4. Conclusions

The application of plasma and high-pressure processing presented good results for orange juice containing prebiotic fructooligosaccharides (FOS). Both processes resulted in some changes in FOS degree of polymerization. Regarding the FOS preservation, HPP showed the highest FOS degradation overall percentage. Higher FOS depolymerization was found in orange juice (10.6 to 21.4 g/100 g) compared to the same processing with only FOS in water (0.5 to 11.0 g/100 g) evidencing the importance of the food matrix interaction. However, the FOS in orange juice submitted to ACP and HPP was well preserved compared to previous studies on thermal treatment (pasteurization), which presented FOS depolymerization from 30 to 100 g/100 g. The treated samples presented a slight variation on the color parameters after both treatments (ACP and HPP). However, the human eye is unable to detect the small differences in the samples treated with plasma and for HPP the color became more vivid. The evaluated processes also did not degrade the main organic acids in orange juice. In fact, the vitamin C content significantly increased due to HPP and slightly increased after plasma treatments. Vitamin C preservation is imperative because orange juice is consumed as a vitamin C source. Regarding the HPP processing, the use of industrial equipment allowed to control the temperature at low levels (~11.5 °C) avoiding any thermal effect. The results obtained in the present study were better than reported for the classical pasteurization, making these technologies suitable and promising for industrial application.

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